PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT-12257	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/03453	International filing date *(day/month/year)* 02.04.2003	Priority date *(day/month/year)* 03.04.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/68		
Applicant PROCORDE GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.10.2003	22.01.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Moreno de Vega, C Telephone No. +49 89 2399-7486

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/03453

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-22 as originally filed

Claims, Numbers

1-21 received on 22.12.2003 with letter of 22.12.2003

Drawings, Sheets

1/9-9/9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 15-19 with respect to industriall applicability

   because:

   ☒ the said international application, or the said claims Nos. 15-19 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14, 20, 21
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 15-19 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D1: LAUGWITZ KARL-LUDWIG ET AL: 'Blocking caspase-activated apoptosis improves contractility in failing myocardium.' HUMAN GENE THERAPY, vol. 12, no. 17, 20 November 2001 (2001-11-20), pages 2051-2063, XP001117410 ISSN: 1043-0342 cited in the application

1. Article 33(2) PCT

D1 discloses a study about the role of caspase activation in cardiac contractility and sarcomere organization in the development of congestive heart failure.
Present claims 1-21 appear to be novel, as the known prior art does not disclose the methods and kits for screening of compounds for the treatment of cardiovascular disease using a ventricular myosin light chain type 1 (vMLC1).

2. Article 33(3) PCT

D1, which is considered to be the most relevant prior art with respect to present claims 1-21, does not disclose that vMLC1 is a target of active caspase-3 and that vMLC1 is cleaved in failing myocardium *in vivo*. The technical problem to be solved by the present invention is the provision of methods and kits for the screening of compounds for the treatment of chronic or acute cardiovascular disease. The solution proposed by present claims 1-21 is based on the finding that direct cleavage of vMLC1 by activated caspase-3 contributes to depression of myocyte function by altering cross-bridge interactions between myosin and actin molecules and that activation of apoptotic pathway in the heart leads to contractile dysfunction prior to cell death. There is no hint in the known prior art to arrive at this solution.
Therefore, claims 1-21 meet the requirements of Article 33(3) PCT

3. For the assessment of the present claims 15-19 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims

1. Use of a peptide containing an essential ventricular myosin light chain type 1 (vMLC1) amino acid sequence, which is functional as cleavage site for caspase-3, in the screening for a compound for the treatment of chronic or acute cardiovascular disease.

2. Use according to claim 1, wherein the amino acid sequence is DFVE.

3. Use according to claim 1 or 2, wherein the peptide is vMLC1.

4. Use according to any one of the preceding claims wherein the screening is directed to a compound which selectively inhibits the caspase-3-mediated cleavage of vMLC1 under predetermined conditions while essentially not inhibiting the caspase-3-mediated cleavage of a protein containing a functional caspase-3 DEVD cleavage site under the same conditions.

5. Use according to claim 4, wherein the selectivity is based on the structure of the compound.

6. Use according to claim 4, wherein the selectivity of the compound is based on the concentration of the compound.

7. A screening method for inhibitors of the caspase-3-mediated cleavage of vMLC1, which comprises:
   (a) contacting a test compound and a sample containing
      (i) a peptide containing a vMLC1 amino acid sequence which is functional as cleavage site for caspase-3, and
      (ii) caspase-3,
      under predetermined conditions allowing cleavage of the peptide at the cleavage site in the absence of the test compound, followed by
   (b) determining the presence or absence of an inhibition of the protein cleavage activity at the cleavage site as compared to the absence of the test compound, and
   (c) identifying a compound as an inhibitor which provides for the presence of inhibition of the caspase-3-mediated cleavage of the protein in step (b).

8. A screening method for selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site, which comprises:

(a) contacting a predetermined amount of an inhibitor identified or identifiable by the screening method of claim 7 and a sample containing

(i) a peptide containing a functional caspase-3 DEVD cleavage site,

(ii) caspase-3, and optionally

(iii) a peptide containing a functional caspase-3 vMLC1 cleavage site,

under predetermined conditions allowing cleavage of a peptide containing a functional caspase-3 vMLC1 cleavage site in the absence of the test compound, followed by

(b) determining the presence or absence of a change of the protein cleavage activity at the cleavage site of the peptide containing a functional caspase-3 DEVD cleavage site as compared to the absence of the test compound, and

(c) identifying a compound as a selective inhibitor which provides at the predetermined concentration for an essential absence of a change of the protein cleavage activity at the cleavage site of the peptide containing a functional caspase-3 DEVD cleavage site.

9. The method of claim 7, wherein the screening method for selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site of claim 8 is simultaneously carried out.

10. The method of any one of claims 7 to 9, wherein the peptide containing a vMLC1 amino acid sequence which is functional as cleavage site for caspase-3 is vMLC1.

11. The method of any one of claims 7 to 10, wherein the peptide contains the sequence DFVE as amino acid sequence of essential ventricular myosin light chain which is functional as cleavage site for caspase-3.

12. A cell assay for screening for inhibitors of the caspase-3-mediated cleavage of vMLC1, which comprises

(a) providing a culture of isolated cardiomyocytes,

(b) introducing activated caspase-3 into cardiomyocytes of step (a),

(c) determining the presence or absence of a reduction of the extent of caspase-3-mediated cleavage of vMLC1 and/or an improvement of cell contractility under predetermined conditions in the presence of a test compound as compared to the absence of the test compound,

(d) identifying a compound as an inhibitor which provides for the presence of inhibition of the caspase-3-mediated cleavage of vMLC1 and/or for an improved cell contractility in step (c).

13. A cell assay for screening for selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site, which comprises

(a) providing a culture of isolated cardiomyocytes,

(b) introducing activated caspase-3 into cardiomyocytes of step (a),

(c) determining the presence or absence of a change of the extent of protein cleavage at the cleavage site of the peptide containing a functional caspase-3 DEVD cleavage site in the presence of a predetermined amount of an inhibitor identified or identifiable by the assay of claim 12 as compared to the absence of the inhibitor, and

(c) identifying a compound as a selective inhibitor which provides in the predetermined amount for an essential absence of a change of the protein cleavage at the cleavage site of the peptide containing a functional caspase-3 DEVD cleavage site.

14. The assay of claims 12, wherein the assay for screening for selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site of claim 13 is simultaneously carried out.

15. An *in vivo* assay for screening for inhibitors of the caspase-3-mediated cleavage of vMLC1, which comprises

(a) providing an animal model, preferably for heart failure,

(b) administering a test compound to the animal model of step (a),

(c) determining the presence or absence of a reduction of the extent of caspase-3-mediated cleavage of vMLC1 and/or an improvement of heart failure under predetermined conditions in the presence of the test compound as compared to the absence of the test compound,

(d) identifying a compound as an inhibitor which provides for the presence of inhibition of the caspase-3-mediated cleavage of vMLC1 and/or for an improvement of heart failure in step (c).

16. An *in vivo* assay for screening for selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site, which comprises

(a) providing an animal model, preferably for heart failure,

(b) administering a test compound to the animal model of step (a),

(c) determining the presence or absence of a change of the extent of protein cleavage at the cleavage site of the peptide containing a functional caspase-3 DEVD cleavage site in the presence of a predetermined amount of an inhibitor identified or identifiable by the assay of one of claims 7 to 15 as compared to the absence of the inhibitor, and

(d) identifying a compound as a selective inhibitor which provides in the predetermined amount for an essential absence of a change of the protein cleavage activity at the cleavage site of the peptide containing a functional caspase-3 DEVD cleavage site.

17. The assay of claims 15, wherein the assay for screening for selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site of claim 16 is simultaneously carried out.

18. The assay of any one of claims 15 to 17, wherein the determination in step (c) is performed based on a measurement of contractility of cardiomyocytes and/or Western blotting.

19. The assay of claim 12 or 15, wherein the reduction in the extent of caspase-3-mediated cleavage of vMLC1 is determined by detection of a specific cleavage product of caspase-3-mediated cleavage of vMLC1, notably by Western blotting.

20. Kit-of-parts for identifying inhibitors of the caspase-3-mediated cleavage of vMLC1 according to claim 7, comprising the following components:

(i) a first component comprising a peptide containing an essential ventricular myosin light chain amino acid sequence, which is functional as cleavage site for caspase-3, and

(ii) a second component comprising caspase-3.

21. Kit-of-parts for identifying selective inhibitors of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site according to claim 8, comprising the following components:

(i) a first component comprising a peptide containing a functional caspase-3 DEVD cleavage site,

(ii) a second component containing caspase-3, and optionally

(iii) a third component comprising a peptide containing a functional caspase-3 vMLC1 cleavage site.

22. Inhibitor of caspase-3-mediated cleavage of essential ventricular myosin light chain obtained or obtainable by the method of any one of claims 1 to 19.

23. The inhibitor according to claim 22, which is a selective inhibitor of the caspase-3-mediated cleavage of vMLC1 over the caspase-3-mediated cleavage of a peptide containing a functional caspase-3 DEVD cleavage site.

24. The inhibitor according to claim 22, which is a peptide containing the sequence DFVE, or a derivative thereof.

25. Use of the inhibitor according to any one of claims 22 to 24 for the preparation of a medicament for the treatment of chronic cardiovascular disease.

26. Medicine containing as an active agent a compound which is characterized by inhibiting caspase-3-mediated cleavage of vMLC1.

27. Peptide containing the sequence DFVE as amino acid sequence of essential myosin light chain which is functional as cleavage site for caspase-3, with the exception of native essential myosin light chain.